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TITLE: SIMIAN HEMORRHAGIC FEVER (SHF) VIRUS

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Phase III of this contract required that we twice-clone selected simian hemorrhagic fever (SHF) virus-specific hybridoma cultures, expand two clones from each clone as well as 50 ml of supernatant fluid from cultures of each clone.

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Final Report for Phase III
Contract No.: DAMD 17-91-C-1006
Principal Investigator: Margo A. Brinton
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Phase III of this contract required that we twice-clone selected simian hemorrhagic fever (SHF) virus-specific hybridoma cultures, expand two clones from each of the selected hybridomas and deliver to USAMRIID 10 vials of frozen cells of each clone as well as 50 ml of supernatant fluid from cultures of each clone. We have completed Phase III as described in detail below.

Production and screening of a second set of hybridomas. The set of SHFV hybridomas described in the Phase I and II Final Reports unfortunately did not continue to produce SHFV-specific antibody after single cell cloning. Therefore, a second set of hybridomas was produced. This time two sets of five mice were each immunized with SHF virions purified by centrifugation through a 15 to 55% sucrose density gradient. The first group of Balb c mice were 3 month old females and each received 3.85×10^7 PFU of SHFV in 100 μl by the subcutaneous route. The second group of Balb c mice were 1 month old females and each received 9.5×10^7 PFU of SHFV in 100 μl by the subcutaneous route. TiterMax (CytRx Corp.) was used as the adjuvant for the first virus injection. A second injection was given on day 32 to the first group of mice and on day 28 to the second group. For this injection mice in the first group were given pelleted virus resuspended in HBSS (9.5×10^7 PFU/100 μl per mouse). Mice in the second group were given a 1:1 mixture of sucrose gradient purified virus and pelleted virus (a total of 7.2×10^8 PFU/100 μl per mouse). Immunized mice were bled approximately 21 days after the second injection and the plasma was tested for SHFV reactivity by ELISA assay at USAMRIID and by Western blotting at Georgia State University. Reactivity to SHFV Vp1, Vp2, and Vp3 proteins was detected in all 10 mice by Western blotting. Specific reactivity to purified virion as well as cell extract SHFV antigen preparations was observed in all mice by ELISA assay. The mouse in each group which showed the best response in both assays was selected as the spleen donor for the production of hybridomas.

Supernatants from the 268 wells in which cells grew were first tested for SHFV reactivity by ELISA. Those supernatants showing the highest levels of reactivity were subsequently tested by Western blotting. The results indicated that 6 of the supernatants reacted with Vp1, 6 of the supernatants reacted with Vp2, 7 of the supernatants reacted with a 35 KD and a 45 KD band in the Vp3 region and 5 of the supernatants reacted with a 35 KD band in the Vp3 region. At least three hybridomas representing each of the four unique reactivity patterns were selected.

Cloning of selected SHF virus specific hybridoma cultures. Western blot reactivity patterns are shown in Figure 1 and ELISA titers are shown in Table 1. The four selected hybridomas, AA4 (Vp2), AD4 (Vp3?), FC2 (Vp1), and HD4 (Vp3) (one for each reactivity pattern), were then subjected to two rounds of single cell cloning to assure the monospecificity and stability of the selected hybridoma cultures. Supernatants from cell positive wells in the single cell cloning dishes were first tested for SHFV reactivity by

ELISA. Approximately 90% of these wells produced antibodies with SHFV-specific reactivity. Those supernatants with the highest ELISA titers were selected (Table 2). In all cases the supernatants from these cloned cells reacted with the same SHFV protein as did the original hybridoma culture from which it was cloned. For each of the four unique reactivity patterns, two clones with good reactivity were selected and subjected to a second round of single cell cloning. Two clones of each reactivity type were again selected using the same methods as described above (Table 3).

Expansion of the selected, cloned hybridomas. The eight clones representing two clones for each of the four reactivity patterns were then expanded. Cells were aliquoted at a concentration of about 10^7 cells per vial and frozen by programmed reduction of temperature. Fifty milliliters of long-term culture fluid for each clone were also obtained. Ten vials of each type of cloned cell (80 vials in total) were shipped on dry ice and the supernatants were shipped on wet ice to Dr. Fred Knauert at USAMRIID.

Additional SHFV hybridomas. Although the shipment of hybridomas and supernatants already received by USAMRIID completely fulfills the contract, we have begun to twice-clone an additional backup hybridoma for each reactivity pattern. The backup set of four hybridomas selected for cloning are BB2 (Vp2), AC1 (Vp1), BB3 (Vp3?), and HC5 (Vp3). These hybridomas will be cloned twice and clones will be selected as described above. Eighty vials of frozen cells and 50 ml of supernatant from each of the 8 clones will then be sent to USAMRIID at no additional cost.

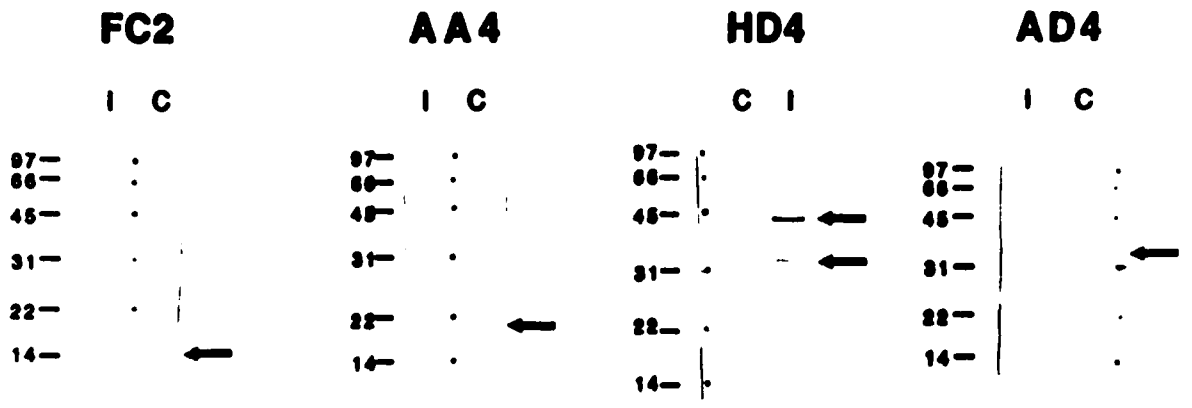

Figure 1. Western blot analysis of four selected SHFV hybridomas. FC2 reacts with Vp1. AA4 reacts with Vp2. HD4 reacts with a 45 KD and a 35 KD band. AD4 reacts with a 35 KD band. Arrows indicate positions of the virus protein bands. The positions of the molecular weight standards are indicated on the left side of each nitrocellulose strip. I - SHFV pelleted from infected tissue culture supernatant. C - uninfected pelleted tissue culture supernatant.

Table 1. The ELISA O.D. values, Western Blot and IgG Dot Blot results from the selected hybridoma supernatants.

Hybridoma	RV204	RV204 titer	SHF-HBSS	SHF-CTRL	WB protein	WB intensity	IgG intensity
AA4	0.60	50	0.00	0.00	VP2	strong	2
AD4	0.51	50	0.00	0.00	unknown		4
FC2	0.55	50	0.00	0.00	VP1	strong	3
HD4	0.49	50	0.00	0.00	VP3	strong	4

Table 2. The ELISA O.D. values from once cloned hybridomas chosen to be cloned a second time.

A.

1st cloning	RV204 1:200	RV193 1:500	Pellet 1000
HD4/AB11	1.68	0.00	0.00
AD4/AA9	1.30	0.00	0.00
AA4/BB5	1.40	0.00	0.00
FC2/AA1	1.18	0.00	0.00

B.

	Dilutions of Supernatants							
1st cloning	5	20	80	320	Titer	Sum	O.D.	Diln.
HD4/AB11	O.59[1]	0.71	0.65	0.48	640	2.46	0.71	20
AD4/AA9	0.57	0.62	0.45	0.31	320	1.94	0.57	5
AA4/BB5	0.55	0.30	0.36	0.38	390	1.60	0.55	5
FC2/AA1	0.24	0.48	0.17	0.16	20	1.50	0.40	20

[1] SHF gradient purified antigen was used at a dilution of 1:200.

Table 3. The ELISA O.D. values from the selected hybridomas after 2nd single cell cloning.

	Dilutions of Supernatants							
2nd cloning	5	20	80	320	Titer	Sum	O.D.	Diln.
AA4/BB5-AC4	0.45[1]	0.37	0.34	0.33	320	1.50	0.45	5
AA4/BB5-AE3	0.48	0.29	0.31	0.24	320	1.30	0.48	5
AD4/AA9-BE3	0.41	0.38	0.30	0.14	80	1.23	0.41	5
AD4/AA9-BF4	0.50	0.44	0.37	0.19	320	1.50	0.50	5
FC2/AA1-AF4	0.44	0.41	0.32	0.23	320	1.40	0.44	5
FC2/AA1-BH2	0.50	0.41	0.28	0.23	320	1.42	0.50	5
HD4/AB11-AA7	0.57	0.45	0.35	0.24	320	1.61	0.57	5
HD4/AB11-AD8	0.51	0.49	0.40	0.31	320	1.72	0.50	5

[1] SHF gradient purified antigen was used at a dilution of 1:200.